Cardiac Output in Fish: Measurement Techniques and Applications

E.D. Linton, D.A. Scruton and R.S. McKinley

Science Branch
Department of Fisheries and Oceans
P.O. Box 5667
St. John's NL Canada A1C 5X1

2004

Canadian Technical Report of
Fisheries and Aquatic Sciences
No. 2555

Fisheries and Oceans Pêches et Océans

Canada

Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of the Department of Fisheries and Oceans, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in *Aquatic Sciences and Fisheries Abstracts* and indexed in the Department's annual index to scientific and technical publications.

Numbers 1–456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457–714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715–924 were issued as Department of Fisheries and the Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page. Out-of-stock reports will be supplied for a fee by commercial agents.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques du ministère des Pêches et des Océans, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications complètes. Le titre exact paraît au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la revue *Résumés des sciences aquatiques et halieutiques*, et ils sont classés dans l'index annual des publications scientifiques et techniques du Ministère.

Les numéros 1 à 456 de cette série ont été publiés à titre de rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre. Les rapports épuisés seront fournis contre rétribution par des agents commerciaux.

105-00897/

Canadian Technical Report of
Fisheries and Aquatic Sciences 2555

2004

CARDIAC OUTPUT IN FISH: MEASUREMENT TECHNIQUES AND APPLICATIONS

by

E.D. Linton[1], D.A. Scruton and R.S. McKinley

Fisheries and Oceans Canada
Science Branch
P.O. Box 5667
St. John's NL A1C 5X1

[1]Dillon Consulting Limited
Cambridge ON N3H 4R7

© Her Majesty the Queen in Right of Canada, 2004.
Cat. No. Fs 97-6/2555E ISSN 0706-6457

Correct citation for this publication:

Linton, E.D., Scruton, D.A. and McKinley, R.S. 2004. Cardiac output in fish: measurement techniques and applications. Can. Tech. Rep. Fish. Aquat. Sci. 2555: iv + 28 p.

TABLE OF CONTENTS

Page

ABSTRACT ..... **IV**

RÉSUMÉ ..... **IV**

INTRODUCTION **1**

MEASUREMENT TECHNIQUES **1**
FICK PRINCIPLE 1
PERFUSED HEART PREPARATIONS 3
INDICATOR DILUTION METHOD 7
FLOWPROBE AFFIXATION TO LIVE, INTACT FISH 8

APPLICATIONS OF CARDIAC OUTPUT MEASUREMENT **12**
INDIRECT ESTIMATE OF METABOLIC RATE 13
AUTOMATED BIOLOGICAL MONITORING 18
CHEMICAL UPTAKE AT THE GILLS 19

CONCLUSION **19**

REFERENCES **19**

ABSTRACT

Linton, E.D., Scruton, D.A. and McKinley, R.S. 2004. Cardiac output in fish: measurement techniques and applications. Can. Tech. Rep. Fish. Aquat. Sci. 2555: iv + 28 p.

This paper reviews the measurement techniques and current applications of cardiac output ($\dot{Q}$) technology in fish. Direct (indicator dilution, perfused heart preparations, flowprobes) and indirect (Fick Principle) methods of measuring $\dot{Q}$ are discussed as well as the advantages and disadvantages of each. The novel applications of $\dot{Q}$ measurement that have more recently emerged, such as in the study of fish metabolism, biomonitoring and chemical uptake at the gills are subsequently outlined.

RÉSUMÉ

Linton, E.D., Scruton, D.A. and McKinley, R.S. 2004. Cardiac output in fish: measurement techniques and applications. Can. Tech. Rep. Fish. Aquat. Sci. 2555: iv + 28 p.

Le présent rapport examine les techniques de mesure du débit cardiaque ($\dot{Q}$) chez les poissons et les applications actuelles de cette technologie. On traite des méthodes directes (dilution d'un indicateur, préparations de coeur perfusé, sondes débitmètres) et indirectes (principe de Fick) de mesure de $\dot{Q}$ ainsi que des avantages et inconvénients de chacune. On brosse ensuite un tableau des applications de la mesure de $\dot{Q}$ récemment apparues, notamment dans les études sur les poissons touchant le métabolisme, la biosurveillance et l'absorption des substances chimiques par les branchies.

INTRODUCTION

Cardiac output ($\dot{Q}$) is an important component of cardiovascular function of all animals. The two variables that determine $\dot{Q}$ are heart rate (f_H; beats min^{-1}) and stroke volume (Vs; ml $beat^{-1}$ kg^{-1}), such that $\dot{Q}$ (ml min^{-1} kg^{-1}) is the product of the frequency of pumping and the volume of blood pumped during each contraction;

$$\dot{Q} = f_H \cdot Vs.$$

The methods used to measure $\dot{Q}$ have evolved significantly over the years such that today blood flow from the heart can be measured accurately and precisely in almost all fish species. With the advent of reliable and quantifiable measurements of $\dot{Q}$, the uses of this technology outside of the study of pure physiology have expanded today to issues of a more applied nature. This paper reviews the techniques used to measure $\dot{Q}$ in fish and the current and some of the emerging applications of $\dot{Q}$ analysis, with emphasis on the emerging novel applications of $\dot{Q}$ technology.

MEASUREMENT TECHNIQUES

Cardiac output can be determined using indirect and direct methods. It can be calculated indirectly using the Fick principle. Alternatively, $\dot{Q}$ can be measured directly using perfused heart preparations or live, intact fish, by employing indicator substances or flowprobe technology. The advantages and disadvantages of each type of $\dot{Q}$ measurement are outlined below. Farrell (1984) also provides an overview of $\dot{Q}$ measurement techniques in fish.

FICK PRINCIPLE

Indirect estimates of $\dot{Q}$ can be made using the Fick equation;

$$VO_2 = \dot{Q} \cdot EO_2,$$

where VO_2 is oxygen consumption (mg $kg^{-1} \cdot h^{-1}$), $\dot{Q}$ is cardiac output (ml $kg^{-1} \cdot h^{-1}$) and EO_2 (mg ml^{-1}) is the difference in arterial and venous blood oxygen contents (Webber et al. 1998). By rearranging the Fick equation, $\dot{Q}$ can be solved for if the other variables are known. The Fick principle is a cost-effective method of determining $\dot{Q}$ and was largely employed before the advent flowprobe technology. It is also a practical method of estimating $\dot{Q}$ in fish such as the channel catfish, *Ictalarus punctatus*, as there is a short distance between the bulbous arteriosus and the first afferent branchial artery that makes flowprobe affixation difficult (McKim et al. 1994).

Estimating VO_2 and EO_2 in fish require both surgical and respirometry equipment. Determining VO_2 commonly entails measuring the dissolved oxygen concentration of water within a respirometer (Cech 1990). The difference in arterial and venous blood oxygen contents is regularly ascertained by directly determining the oxygen levels in blood samples taken from cannulated dorsal and ventral aortas (Kiceniuk and Jones 1977; Metcalfe and Butler 1982; Neumann et al. 1983). The dorsal aorta carries oxygenated blood away from the respiratory surface of the gills to the arterial system whereas the ventral aorta carries deoxygenated blood from the heart to the gills through the venous system. Once VO_2 and EO_2 are known, $\dot{Q}$ can be solved for. The components of $\dot{Q}$ can be determined by placing electrodes for electrocardiogram (ECG) recording adjacent to the pericardial cavity in order to measure f_H. Stroke volume can subsequently be calculated by dividing $\dot{Q}$ by f_H (Kiceniuk and Jones 1977). Calculating $\dot{Q}$ using the Fick equation has been criticized because the Fick principle overlooks some very important physiological functions, namely, prebranchial shunting, gill oxygen consumption and cutaneous oxygen uptake.

Criticisms of the Fick Principle

The Fick principle has been criticized for its neglect to account for blood flow shunts away from the respiratory surface of the gills. The gills of some fish species house both respiratory and non-respiratory blood pathways, meaning that a proportion of the blood entering the gills returns directly to the heart via the venous circulation in the gills and head without oxygenation. This event is referred to as prebranchial shunting (Richards and Fromm 1969; Vogel et al. 1976; Dunel and Laurent 1980; Cooke 1980; Cooke and Campbell 1980; Olson and Kent 1980; Hughes et al. 1982). For example, Hughes et al. (1982) reported that in the European eel (*Anguilla anguilla*) 28% of the venous blood afferent to the gills was returned directly to the heart without passing the respiratory surface of the gill lamella. Cardiac output will be underestimated if blood diversions away from the gas exchange surface are not accounted for in applicable species (Metcalfe and Butler 1982; Neumann et al. 1983).

The Fick principle assumes that all oxygen removed from the water enters the blood and is transported away from the gills, which overlooks the aerobic requirements of the gills. Various cells within the gill tissues require oxygen for waste excretion, ion exchange, chemoreception, mucosal and chloride secretion and contraction (Johansen and Peterson 1981). Oxygen consumption in excised, perfused cod *(Gadus morhua)* gills is approximately 7% of the total VO_2 (Johansen and Peterson 1981). The oxygen requirements of the gills in blood-perfused rainbow trout (*Oncorhynchus mykiss*) preparations ranges from 19-75% of the total VO_2 (Daxboeck et al. 1982). When oxygen consumption by the gill tissue is not included in calculations of $\dot{Q}$ using the Fick principle, the calculated value for $\dot{Q}$ will be larger than the actual value (Metcalfe and Butler 1982; Neumann et al. 1983).

Cutaneous oxygen uptake is disregarded by the Fick principle, but can account for a substantial proportion the total oxygen uptake in fish. Cutaneous oxygen uptake contributes to total VO_2 by 40% in Antarctic ice fish (*Chaenocephalus aceratus*) (Hemmingsen and Douglas 1970), 35% in European eels, 13% in rainbow trout (Kirsch and Nonnotte 1977) and 20% in dogfish (*Squalus acanthias*) (Nonnotte and Kirsch 1978). The Fick principle can overestimate $\dot{Q}$ if cutaneous oxygen uptake is not taken into account (Metcalfe and Butler 1982; Neumann et al. 1983).

Farrell and Jones (1992) summarized that the Fick principle can either over- or underestimate $\dot{Q}$ by up to 40%. Metcalfe and Butler (1982) reported that in perfused dogfish hearts subject to normoxic or hypoxic conditions directly measured $\dot{Q}$ values were similar or 38% higher, respectively, to those calculated using the Fick equation. In rainbow trout heart preparations, Daxboeck et al. (1982) noted that Fick equation calculated $\dot{Q}$ to be 1.5 and 1.8 times higher during normoxia and hypoxia, respectively, than the actual value set by a mechanical pump. Holeton and Randall (1967) found that in rainbow trout the $\dot{Q}$ values calculated using the Fick equation were nearly an order of magnitude higher than exact quantitative measurements. In exercising and recovering rainbow trout, Neumann et al. (1983) determined that the Fick equation and two indicator dilution techniques yielded similar $\dot{Q}$ values, suggesting that both methods had the same margin of error or that the sources of error inherent to the Fick principle were not significant or quantifiable. Thorarensen et al. (1996a) found that directly measured maximum $\dot{Q}$ ($\dot{Q}_{max}$) values in rainbow trout were similar to those calculated using the Fick equation by Kiceniuk and Jones (1977). This finding supported the view of Randall (1985) that errors inherent in the Fick equation cancel out. Conversely, the $\dot{Q}_{max}$ values that Farrell et al. (1991) directly measured in perfused heart preparations were slightly higher than the $\dot{Q}_{max}$ values calculated by Kiceniuk and Jones (1977). These examples illustrate that calculating $\dot{Q}$ using the Fick equation produces unpredictable and often inaccurate results. Researchers today often employ direct measures of estimating $\dot{Q}$, as such techniques are less prone to the intrinsic challenges that plague the Fick principle.

PERFUSED HEART PREPARATIONS

Perfused heart preparations have been used to study cardiac physiology in many fish species with great success, as there are numerous advantages to their use. Heart preparations provide a controlled experimental setting (i.e. input and output pressures, $\dot{Q}$ and Vs can be precisely controlled) and therefore allow isolated cardiac parameters to be studied without involving the homeostatic mechanisms present in intact animals (Perry and Farrell 1989). Heart preparations also serve as an excellent alternative to experimenting with live, intact fish, as large and elaborate equipment is not compulsory. Although not well suited for descriptive studies, heart preparations are often used to elucidate mechanisms of known physiological processes that are difficult to study in intact animals (Perry and Farrell 1989). For example, studies using heart preparations

have furthered the understanding of myocardial VO_2 (Graham and Farrell 1989; Farrell and Jones 1992) and cardiac hormone release (Cousins and Farrell 1996).

There are some fundamental components involved in using perfused heart preparation to measure $\dot{Q}$. Input and output cannulae need to be inserted into anterior and posterior locations in the cardiac unit, respectively, and connected to adjustable pressure devices that precisely control the filling and output pressures (Perry and Farrell 1989; Farrell et al. 1992). The input cannula is also connected to a perfusate reservior (Farrell et al. 1986; Perry and Farrell 1989). During surgery there should be minimal disruption of flow to the heart and handling of the cardiac tissues (Farrell et al. 1986; Graham and Farrell 1989). Cardiac output is a function of the input pressure, which dictates Vs, and the pacing frequency (Perry and Farrell 1989). It can be calculated by collecting the perfusate from the output cannula for a specific length of time and quantifying the amount collected by weight or volume (Tort et al. 1987; Farrell et al. 1989).

Cardiac output is more frequently measured using an instrument called a flowprobe. Flowprobes directly measure the volume of blood flow and/or blood velocity and can be connected directly or in-series with the output cannula (Graham and Farrell 1989; Farrell et al. 1992; Franklin and Davie 1993). Flowprobes are sold with accompanying flowmeters that transduce the signal received by the flowprobe into usable output data. It is prudent to verify the calibration of manufacturer-calibrated flowprobes (i.e. Transonic) and to calibrate other types of flowprobes (i.e. electromagnetic, Doppler and thermal) so that data can be expressed as absolute flow rates. Ideally, calibrations should be performed *in situ* at the experimental temperature using several physiologically relevant flow rates of the experimental perfusate. Data can be recorded using a computer equipped with a data acquisition system (Laboratory Technologies Corporation, Wilmington, MA; National Instruments, Austin, TX) (Cousins and Farrell 1996; Axelsson et al. 1992, 2000; Linton, 2003) and Labview® (National Instruments, Austin, TX) (Cooke et al. 2001; Linton, 2003) or AcqKnowledge (Biopac Systems, Inc., Goleta, CA) software. Alternatively, Grass chart recorders (Grass Instruments, Quincy, MA) (Thorarensen et al. 1996a; Axelsson et al. 2000) or Gould strip-chart recorders (Gould, Cleveland OH) (Farrell et al. 1988; Davie and Franklin 1992; Keen and Farrell 1994; Stecyk and Farrell, 2002) can be used for data recording. The accurate measurement of $\dot{Q}$ depends on the quality of the heart preparation, which is largely dictated by the degree to which physiological conditions, pericardium integrity and coronary perfusion can be maintained.

Factors Influencing Preparation Quality

Simulation of Physiological Conditions: It is important that perfused heart preparations reflect *in vivo* conditions as closely as possible so that results may be meaningfully interpreted and compared between studies (Farrell et al. 1989). At one time, Farrell (1984) criticized heart preparations for their "apparent disregard for physiological conditions *in vitro*". Today, heart preparations closely mimic *in vivo* physiological characteristics. The perfusate of choice is whole blood from the same

species as that used in experimentation, but it may be costly or laborious to obtain sufficient amounts of blood for perfusion (Perry and Farrell 1989). Beef, pig or other animal blood can be used as perfusate (Cooke et al. 2001, 2003a; Schreer et al. 2001), but saline fortified with constituents that enhance its physiological resemblance to whole blood (i.e. buffering ability, oxygen carrying capacity, viscosity and gas, ion, metabolite and hormonal composition) is most commonly employed (Perry and Farrell 1989; Graham and Farrell 1989; Davie et al. 1992). Nanomolar concentrations of adrenaline similar to those circulating in the blood of resting fish are customarily added to perfusate to provide tonic cardiac stimulation and improve the preparation's longevity (Graham and Farrell 1989; Keen and Farrell 1994). Heart rate is set by the pacemaker if it is intact, but is modulated by adrenaline, which elicits positive inotropic and chronotropic effects (Graham and Farrell 1989; Farrell et al. 1991; Axelsson et al. 1992; Keen and Farrell 1994). Other manipulated components of a heart preparation, such as the input and afterload pressures, maximum cardiac performance and pacing frequency, should also reflect *in vivo* conditions (Farrell et al. 1989).

Pericardium Integrity: Maintaining an intact pericardium or musculoskeletal framework in perfused heart preparations is essential for the accurate measurement of $\dot{Q}$. Aquatic organisms with a thick pericardial membrane (elasmobranchs) or those with a thin pericardium that covers a rigid musculoskeletal framework (active teleosts; tuna, kingfish, some salmonids) are capable of vis-à-fronte atrial filling (Farrell and Jones 1992; Franklin and Davie 1993). Vis-à-fronte atrial filling directly utilises some of the energy of ventricular contraction to distend the atrium, which induces a negative suction pressure in the pericardium and assists in filling the lumen of the atrium (Farrell and Jones 1992). When the pericardium or musculoskeletal framework is absent or damaged, vis-à-fronte atrial filling is hindered because subambient intrapericardial pressure cannot be fully established in the cardiac cavity (Farrell and Jones 1992). Atrial filling in elasmobranchs and active teleosts is achieved by both vis-à-fronte and vis-à-tergo (force from behind) mechanisms (Farrell and Jones 1992); the former is responsible for the achievement of the first 50-65% of Vs and the latter is integral in producing maximum Vs (Farrell et al. 1988; Farrell and Jones 1992). Opening the pericardium reduces routine $\dot{Q}$ by 55% in dogfish (Franklin and Davie 1993), 44-70% in rainbow trout (Farrell et al. 1988; Keen and Farrell 1994) and $\dot{Q}$max by 18% in rainbow trout (Farrell et al. 1988). Removing the pectoral musculoskeletal framework surrounding the heart decreases Vs by 30% and overall $\dot{Q}$ by 33% in carp (*Cyprinus carpio*), however there are no further changes in $\dot{Q}$ when the pericardium is subsequently cut (Shima and Namba 1996). The $\dot{Q}$ of intact rainbow trout (Kiceniuk and Jones 1977) compares well with that measured in in situ preparations where the pericardium is intact (Keen and Farrell 1994). Overall, *in situ* preparations with an intact pericardium and/or musculoskeletal framework produce accurate cardiac data that can be compared and extrapolated to *in vivo* situations with confidence (Farrell et al. 1988).

Coronary Perfusion: Perfusing the coronary blood vessels is important for maintaining the contractility, tonus and performance of the cardiac unit (Davie et al. 1992; Farrell and Jones 1992). For example, $\dot{Q}$ reaches only 63% of *in vivo* values

when coronary perfusion is absent in perfused skipjack tuna hearts (Farrell et al. 1992). Coronary circulation is present in three of the four ventricular types in fish (Farrell and Jones 1992). It supplements venous oxygen supplies in fish species with powerful, thick ventricle walls where there are physical limits to oxygen diffusion from the lumen to the outer myocardium (Farrell 1984). Such fish species include elasmobranchs and active teleosts such as tuna, trout and salmon (Santer 1985). Fish morphology and the small diameter of the coronary arteries (1-2 mm in 1-2 kg fish) can make perfusing the coronary circulation technically arduous (Farrell et al. 1992; Axelsson and Farrell 1993). Nonetheless, coronary artery perfusion has been accomplished by some researchers (Davie and Farrell 1991; Davie et al. 1992). When the coronary circulation cannot be perfused, increasing the perfusate's oxygen concentration can improve the cardiac performance in small fish (< 1 kg) with thin ventricle walls (Perry and Farrell 1989; Keen and Farrell 1994). All of the above factors should be taken into consideration when working with either type of perfused heart preparation; the *in vitro*, or isolated heart preparation, and the *in situ* preparation.

Types of Perfused Heart Preparations

In Vitro Perfused Heart Preparations: Isolated or *in vitro* heart preparations require that the atrium, ventricle and ventral aorta are completely removed from the body of the fish and immersed in an aerated, temperature-controlled saline bath (Tort et al. 1987; Farrell et al. 1989; Davie and Farrell 1991; Davie et al. 1992). The heart is perfused by inserting an input cannula into the sino-atrial junction and an output cannula into the ventral aorta. The isolation procedure usually excludes some important cardiac tissues such as the sinus venosus. A one-way valve can be inserted into the input cannula to avoid backflow during atrial contractions if the sino-atrial valve is lost during cardiac excision (Perry and Farrell 1989). If the sino-atrial node (where the pacemaker is located) is lost, the ventricle will continue to contract but at a slower, more irregular intrinsic rate relative to intact fish (Farrell 1984; Tort et al. 1987; Perry and Farrell 1989; Davie et al. 1992). Electrical pacing is thus recommended to reduce the variability between heart preparations, but it can obscure induced changes in f_H (Farrell 1984; Perry and Farrell 1989). Pericardium integrity is often disrupted when preparing an *in vitro* heart preparation, although an isolation procedure that does not puncture the pericardium has been described for long-finned eels (*Anguilla dieffenbachii*) (Davie et al. 1992). *In vitro* heart preparations generally perform for less than one hour (Farrell et al. 1992). Many of the technical challenges encountered with isolated heart preparations are overcome with *in situ* preparations.

In Situ Heart Preparations: To prepare an *in situ* heart perfusion a fish is anaesthetized and transferred it to an operating sling where its gills are perfused and the internal organs and intestines are excised (Farrell et al. 1982 1986; Franklin and Davie 1993; Keen and Farrell 1994; Cousins and Farrell 1996). The heart is customarily perfused by cannulating the sinus venosus via the central hepatic vein (Farrell et al. 1986; Perry and Farrell 1989). The output cannula is inserted into the ventral aorta via the mouth once the gills are removed (Cousins and Farrell 1996) or, more frequently, via an

incision in the isthmus, which enables the ventral aorta to be exposed outside of the pericardium (Farrell et al. 1988; Axelsson and Farrell 1993). Veins returning to the heart and sinus venosus are ligated and the cardiac branch of the vagus nerve is crushed or severed (Keen and Farrell 1994; Farrell et al. 1986; Perry and Farrell 1989). Following surgery, the body of the fish is immersed in a controlled-condition (temperature, pH, oxygen) saline bath, ventral side up (Graham and Farrell 1989; Franklin and Davie 1993; Keen and Farrell 1994).

In situ preparations are superior over *in vitro* perparations for a variety of reasons. The pericardium and the sinus venosus can be left intact, enabling vis-à-fronte atrial filling and intrinsic f_H to be maintained, respectively (Farrell 1984; Perry and Farrell 1989; Franklin and Davie 1993). It has been observed, however, that f_H in *in situ* preparations can be slightly greater than resting f_H in intact fish at the same water temperature (Farrell et al. 1982). This type of preparation minimises cardiac tissue handling, retains intrinsic physiological mechanisms and performs at physiological workloads for up to two hours (Farrell et al. 1982, 1983). Furthermore, in situ preparations are applicable to the study of f_H effectors (Farrell 1984; Perry and Farrell 1989).

The quality of an *in situ* heart preparation can be judged by its ability to accurately reproduce *in vivo* physiological conditions (Perry and Farrell 1989). *In situ* perfused heart preparations achieve comparable $\dot{Q}$ values to those observed *in vivo* in resting (Farrell et al. 1982 1983) and exercising fish (Farrell et al. 1992; Keen and Farrell 1994). Indeed, techniques that enable the preparation of physiologically robust heart preparations are well established and widely used to produce accurate, comparable $\dot{Q}$ data over a range of metabolic scopes. Measuring $\dot{Q}$ using perfused heart preparations is advantageous to using live fish because experimental results are not influenced by the stress imparted on the fish by surgery, anaesthetic, handling and experimental confinement. However, measuring $\dot{Q}$ in live fish has the advantage of using intact, innervated, naturally perfused hearts.

INDICATOR DILUTION METHOD

The indicator dilution method of estimating $\dot{Q}$ involves rapidly injecting a known amount of indicator substance into the venous blood and then continuously measuring its concentration in blood sampled from the arterial system (Nichols and O'Rourke 1990). The greater the $\dot{Q}$, the more rapidly the indicator substance is diluted in the arterial blood (Nichols and O'Rourke 1990). The formula commonly used to calculate $\dot{Q}$ is:

$$\dot{Q}\ (\text{l min}^{-1}) = \frac{\text{amount of indicator injected (mg)} \cdot 60\ \text{(s)}}{\text{average arterial concentration (mg l}^{-1}) \cdot \text{duration of curve (s)}}$$

average arterial concentration (mg l^{-1}) · duration of curve (sec) As this method entails affixing live, intact fish with cannulae, fish restraint may be required so that the cannulae do not become dislodged. Suitable indicators do not alter circulation dynamics, do not disappear from the blood and can be quantified in the blood; dye or radioactively labelled substances are most commonly employed (Neumann et al. 1983; Barron et al. 1987; Nichols and O'Rourke 1990). Successful measurement of $\dot{Q}$ using this method also requires that the indicator substance rapidly mixes with the blood and that most of the $\dot{Q}$ flows past the sampling site (Guyton et al. 1973).

Neumann et al. (1983) estimated $\dot{Q}$ in rainbow trout using two different methods. Radioactively labelled microspheres were injected into the dorsal aorta in one set of experiments, while the other technique entailed injecting radioactively labelled inulin into the caudal vein. Known volumes of blood were sampled from the caudal artery every minute and radioactivity was determined using a scintillation counter for both sets of experiments. Inulin diffusion into the extracellular space was a source of error in the calculation of $\dot{Q}$, however blood circulated from the caudal vein to the caudal artery in less than thirty seconds, suggesting that outward diffusion was negligible. The microsphere method estimated $\dot{Q}$ less the volume of blood supplied to the head and shunted towards the venous system pre- or post-branchially. The $\dot{Q}$ values calculated using both methods were comparable and deemed to be reasonable methods of estimating $\dot{Q}$ (Neumann et al. 1983). Barron et al. (1987) used Evan's blue dye in 1% saline as the indicator substance in rainbow trout. They injected the dye solution intracardially, which enabled rapid mixing, and sampled blood from the dorsal aorta. The concentration of dye in each blood sample was determined using a spectrophotometer and the area under the calibration curve of dye concentration *versus* sampling time was calculated and corrected for dye recirculation. Cardiac output was calculated by taking the product of the amount of dye added, area under the calibration curve and fish mass (Guyton et al. 1973).

The indicator dilution method of estimating $\dot{Q}$ in live, intact fish is cost effective compared to using flowprobes. However, ease of use and accuracy of the results make flowprobe affixation in live fish a popular method of measuring $\dot{Q}$.

FLOWPROBE AFFIXATION TO LIVE, INTACT FISH

Affixing the Ventral Aorta with a Flowprobe

To measure $\dot{Q}$ in live, intact fish, flowprobes are affixed to the ventral aorta, the artery carrying all blood from the heart. Fish are anaesthetised before flowprobe surgery and their gills are perfused with a low-dose anaesthetic solution during surgery. The lead wire from the flowprobe should be sutured to one side of the fish's body (Korsmeyer et al. 1997; Thorarensen et al. 1996a; Linton, 2003) or secured under the skin (Webber et al. 1998). If care is taken, none of the variations of flowprobe surgery detailed below should cause haemorrhaging or disrupt the integrity of the pericardium. Flowprobe

surgery can be completed within twenty minutes by a skilled surgeon. There are various methods of fitting teleosts with $\dot{Q}$ flowprobes that vary in their degree of invasiveness.

Transcutaneous Placement: Flowprobes with a flat, square sensing window can be placed transcutaneously over the ventral aorta with adhesive tissue or tape (Bushnell et al. 1990; Korsmeyer et al. 1997). This is the least invasive and fasted method of affixing a fish with a cardiac output flowprobe. The anatomy of tuna lends itself to transcutaneous, rather than surrounding cuff-style flowprobe affixation, because the ventral aorta is surrounded by highly vascularised tissue that haemorrhages excessively when disturbed (Bushnell et al. 1990).

Direct Placement: Flowprobes are most commonly directly applied to the ventral aorta, being cuffed around or cannulated into the vessel. Accessing the ventral aorta necessitates that the operculum and gills on one side of the head are lifted. A tiny incision is made in the overlying layers of thin connective tissues, which are then teased away to completely expose several millimetres of the ventral aorta (Thorarensen et al. 1996a; Brodeur et al. 2001a; Stecyk and Farrell 2002). Alternatively, the ventral aorta can be accessed via an incision along the ventral mid-line of the isthmus and subsequently retracting the white muscle and connective tissues (Kolok et al. 1993; Axelsson et al. 2000; Crocker et al. 2000). Such an incision has the potential to become infected and impair ventilatory control by damaging the muscles and nerves used to open the lower jaw (Bushnell et al. 1990).

Extracorporeal Circulations: Extracorporeal circulations shunt blood from the circulatory system through a tubing assemblage that is external to the body. The partial externalisation of the circulatory system is advantageous in situations that do not lend themselves to the direct use of flowprobes. For example, the ventral aorta in elasmobranchs branches immediately after the conus arteriousus ends, making flowprobe affixation difficult (Davie and Farrell 1991). After attempts to measure $\dot{Q}$ in situ, Hughes et al. (1982) measured $\dot{Q}$ using an extracorporeal circulation so that a constant relative position between the vessel and the transducer could be maintained.

Thermal, electromagnetic, Doppler and Transonic flowprobes have been used to directly measure blood velocity or flow in the ventral aorta and therefore estimate $\dot{Q}$. These types of flowprobes will be discussed in increasing order of their ability to measure $\dot{Q}$ with accuracy and precision.

Types of Flowprobes

Thermal Flowprobes: Thermal flowprobes are classified as intravascular because they catheterise the vessel. They quantify blood flow by measuring the resistance change of a resistor that is heated by an electrical current and cooled by blood flow. The quantifiable change in resistance is flow-dependent (Davie and Forster 1980). Thermal

flowprobes need to be calibrated by the researcher if flow data are to be expressed in absolute terms (Davie and Forster 1980). Flow velocity is estimated using thermal flowprobes, rather than the blood flow volume, the latter being a more accurate method of determining $\dot{Q}$.

Electromagnetic Flowprobes: Electromagnetic flowprobes (Biotronex, Zepeda Instruments, Seattle, WA) measure blood flow using Faraday's law of electromagnetic induction (Reneman and Hoeks 1977; Nichols and O'Rourke 1990). Blood is a conductor of electricity, hence its velocity may be measured by aligning a blood vessel across a magnetic field and recording the induced voltage or current (Nichols and O'Rourke 1990). When the strength of the magnetic field and the vessel diameter are assumed to remain constant, the voltage created varies linearly with flow velocity. Extravascular and intravascular (catheterising) electromagnetic flowprobes exist (Nichols and O'Rourke 1990).

Electromagnetic flowprobes have several shortcomings. Blood velocity, rather than volume flow, is measured, which is a crude estimate of $\dot{Q}$. Additionally, this method assumes that vessel diameter remains constant. The flow signal can be disturbed by electrical interference, such as that from the ECG (Reneman and Hoeks 1977; Nichols and O'Rourke 1990; Transonic Systems Inc. 2003). Electromagnetic flowprobes need to precisely fit the vessel to enable electrical contact between the flowprobe and the vessel, which often results in vessel constriction (Nichols and O'Rourke 1990; Transonic Systems Inc. 2003). Hughes et al. (1982) discontinued the use of electromagnetic flowprobes in eels as they did not have a consistently snug fit around the ventral aorta and shifted position during experimentation and calibration. The main shortcoming of electromagnetic flowmeters is in the precise determination of when zero flow is achieved. The most reliable methods of determining the signal at zero flow *in vivo* are by occluding blood flow, without the vessel wall falling away from the flowprobe, or inducing bradycardia (Nichols and O'Rourke 1990; Transonic Systems Inc. 2003). Accurate calibration necessitates that the zero flow signal is determined.

Doppler Flowprobes: Doppler flowprobes (Iowa Doppler Products, Iowa University, Iowa; Titronics Medical Instruments, Iowa City, Iowa) emit ultrasound waves from a vibrating crystal through a vessel wall and into the blood stream. The frequency of the ultrasound that is backscattered from stationary surfaces (i.e. the opposite vessel wall) is the same as the emitted frequency. However, the ultrasound that is backscattered from moving particles (i.e. erythrocytes) is shifted from the emitted frequency by an amount proportional to the velocity of the particles (Reneman and Hoeks 1977). The crystal can be mounted in a plastic casing for transcutaneous or perivascular use, or in a catheter for intravascular applications (Reneman and Hoeks 1977; Nichols and O'Rourke 1990). Doppler flowprobes can also be constructed by purchasing piezoelectric crystals (Crystal Biotech, Hopkinton, MA) and positioning them in the desired housing (Gamperl et al. 1994).

These flowprobes offer several advantages over the other types of flowprobes discussed above. Doppler flowprobes have a built-in zero indicator (Farrell and

Jones 1992), thus provide reliable information about zero flow (Axelsson et al. 1992). They are preferred when measuring blood flow in vessels of very small diameter, as they are less bulky than electromagnetic (Axelsson et al. 1992) and Transonic flowprobes, and can be obtained in cuff sizes as small as 0.5 mm in diameter (Iowa Doppler Products, 2003). Furthermore, the lead wire from Doppler flowprobes is thinner and lighter relative to other types of flowprobes, causing minimal drag in swimming fish (Thorarensen et al. 1993). The end of the lead wire can be ordered with a thin wire terminus or a bulky end connector (Iowa Doppler Products, 2003). Wire terminus' can easily be fed out of most experimental chambers for subsequent connection to the flowmeter, omitting the need to create large portals in experimental equipment. This is the only type of flowprobe that can measure $\dot{Q}$ non-invasively via transcutaneous application (Reneman and Hoeks 1977).

Although Doppler flowprobes are superior for many reasons, there are some key disadvantages to their use. The main shortcoming intrinsic to Doppler flowprobes is that blood velocity, rather than volume flow, is directly determined (Thorarensen et al. 1993; Nichols and O'Rourke 1990; Transonic Systems Inc. 2003.) Additionally, care must be taken to choose a cuff size that allows the vessel to distend during systole yet minimally shifts in placement and orientation along the ventral aorta, which can alter the signal (E.D. Linton, unpublished observations). Successive calibrations can yield different results even when extreme care is taken, thus the average of several calibrations is recommended. Calibration difficulties in addition to inherent inadequacies with the design of Doppler flowprobes make accurate quantitative information difficult to obtain (Reneman and Hoeks 1977). As a result, $\dot{Q}$ data is often expressed in relative (percent change from basal or initial values) rather than absolute terms (Axelsson et al. 1992, 1994; Brodeur et al. 1999). Doppler flowprobes that are properly cared for can be used an average of ten times before the signal degradation warrants that the flowprobe should be discarded (S. J. Cooke, Pers. Comm). The short life of Doppler flowprobes is counterbalanced by their considerably lower cost than the other widely used type of flowprobe, the Transonic flowprobe.

Transonic Flowprobes: Transonic flowprobes (Transonic Systems Inc., Ithaca, NY), like Doppler flowprobes, measure blood flow using ultrasound waves. The difference is that Transonic flowprobes use transit time ultrasound technology to measure absolute volume flow by calculating the difference in time taken for the ultrasound to pass through the vessel in one direction as opposed to the other. A roughly cuff-shaped flowprobe is fit non-constrictively around the vessel. On one side of the cuff is the probe body that houses two ultrasonic transducers, and at a fixed distance opposite the probe body is a metal plate that reflects the emitted ultrasound. Both transducers can emit and receive ultrasound, and do so in an alternating fashion. For example, the transducer downstream of the blood flow emits a wide wave of ultrasound diagonally into the vessel. The ultrasound travels through the vessel in the upstream direction, bounces off the reflector place, travels through another upstream area of the vessel and is received by the upstream transducer. The same sequence is repeated, except ultrasound is emitted from the transducer upstream of the blood flow, thus ultrasound travels with blood flow in the downstream direction, travelling faster than when moving in the upstream direction

(Transonic Systems Inc. 1999). The difference between the upstream and downstream transit times of the ultrasound gives an estimate of average velocity, which is multiplied by the vessel's cross sectional area to yield volume flow measurements (Transonic Systems Inc. 1999, 2003). Volume flow calculations are independent of vessel diameter and shape (Drost 1978; Transonic Systems Inc. 1999).

Flowprobes from Transonic Systems Inc. are the gold standard in blood flow measuring equipment. They yield accurate, high-resolution flow data and provide readings of absolute and zero blood flow (Thorarensen et al. 1996a). Transonic flowprobes are calibrated by the manufacturer using both blood and water at one temperature. Timely and often tedious *in situ* calibrations for each fish are unnecessary, yet manufacturer calibrations should be verified, particularly if the experimental temperature differs from that of calibration (Franklin and Davie 1993; Kolok et al. 1993). Flow data can, however, be corrected by approximately 2% for every 5 ^{o}C temperature change (Margo Sosa, Transonic Systems Inc., pers. comm.). Each flowprobe comes with a key that is unique to its manufacture and calibration and must be inserted into the flowmeter before signal recording can commence. Transonic flowprobes fit non-constrictively around the vessel, thus the impact of implant on the vessel is reduced (Drost 1978) and the vessel is not constricted. Flowprobes and flowmeters from Transonic Systems Inc. can be used for many years if properly cared for.

Perivascular flowprobes, the type of Transonic flowprobe used to measure $\dot{Q}$ in fish, have many unique features. Various flowprobe configurations are available, allowing researchers to choose the probe that best fits their application and the morphology of the experimental subjects. The location of cable exit from the probe body can be selected from back, side or lateral. Reflector plates also come in a variety of shapes including 'L', 'J' and 'U'; the former two are usually equipped with a sliding cover that encloses the vessel in the probe window.

There are some drawbacks to using Transonic flowprobes to measure blood flow. Transonic flowprobes and the associated flowmeter are of a significantly greater cost than all other types of blood flow measuring equipment. Furthermore, they are the bulkiest of all flowprobe types. The connectors at the end of the cable are bulky, which may necessitate that larger portals are made in the experimental apparatus so that the cable can be fed out. Furthermore, the lead or cable is quite thick and may cause drag in swimming fish (Thorarensen et al. 1993).

APPLICATIONS OF CARDIAC OUTPUT MEASUREMENT

Until recently, the majority of studies examining $\dot{Q}$ have focused on comparative and environmental physiology, of which there are numerous of published accounts. Examples of comparative studies include the examination of cardiac responses of three fish species with different winter activity levels to anaerobic exercise (Cooke et al. 2003a), of riverine and lacustrine rock bass (*Ambloplites rupestris*) to silt loading (Bunt et al. 2004) and of different tuna species to hypoxia (Bushnell et al. 1990).

Research focusing on environmental physiology has examined how $\dot{Q}$, f_H and Vs respond to common environmental variables such as temperature (Barron et al. 1987; Keen and Farrell 1994; Schreer and Cooke, 2002), pH (Brodeur et al. 1999), hypoxia (Davie et al. 1992; Stecyk and Farrell, 2002), hypercapnia (Crocker at al. 2000) and silt (Bunt et al. 2004). More recently, however, $\dot{Q}$ technology has been used to provide insights into issues of a more applied nature (Cooke et al. 2002a), such as fisheries management and pollution monitoring. The novel applications of $\dot{Q}$ technology in fish will be considered in the following sections.

INDIRECT ESTIMATE OF METABOLIC RATE

Cardiac output is a correlate of metabolism, thus it has been applied to a number of unique environmental situations to aid in the assessment of metabolic changes that may result from exposure to environmental stressors. Metabolism is a component of the intricately balanced bioenergetics budget of fish, which takes the form of the following steady-state energy equation;

$$C = M + G + W,$$

where the total amount of energy consumed (C), is balanced by the total mount of energy expended through metabolism (M; active, standard and specific dynamic action), growth (G; somatic and germ cell) and waste (W; egestion and excretion) (Adams and Breck 1990). Assessing changes in metabolism therefore provides insight into the effects of such changes on other energetic parameters. Changes in metabolism can also act as an early warning system to identify sublethal stress before it manifests into lethal or irreversible effects on individuals or populations (Widdows and Donkin 1991).

Fish metabolism has traditionally been determined using respirometry. The pioneer laboratory studies of Brett (1964) used sockeye salmon (*Oncorhynchus nerka*) to generate the paradigm that $\dot{V}O_2$ is a suitable estimate of overall metabolism when anaerobic contributions are insignificant (Cech 1990). The terms metabolic rate and $\dot{V}O_2$ are therefore customarily used interchangeably. Directly measuring the $\dot{V}O_2$ of fish in a respirometer is a tried and proven method of assessing metabolism, although it often only has applications in laboratory research. Transporting respirometers to remote field locations has enabled fish respirometry to be studied outside of the traditional laboratory setting (Farrell et al. 2003; Linton, 2003), however the fish must be confined in a chamber, which is not always possible or desirable in field studies. As a result, indirect methods of measuring metabolism have been explored.

Physiological variables that have been used to indirectly assess $\dot{V}O_2$ include $\dot{Q}$ (Webber et al. 1998), heart rate (Lucas 1994), locomotor muscle activity (Hinch et al. 1996), ventilation rate (Rogers and Weatherly 1983), swimming behaviour (Young et al. 1972) and tailbeat frequency (Johnstone et al. 1992) (refer to Linton (2003) for review). The aforementioned variables, with the exception of $\dot{Q}$, can be monitored using biotelemetry devices, which have applications in field as well as laboratory

research. Cardiac output flowprobes still need to be hardwired, or tethered, to the recording equipment, consequently their use is limited to laboratory studies. However, a blood flow telemetry tag is currently being researched and developed (Cooke et al. 2002b; Axelsson 2004).

There are various advantages and disadvantages associated with using $\dot{Q}$ as an indirect measure of metabolic rate. Recalling the Fick equation ($VO_2 = [f_H \cdot Vs] \cdot EO_2$), an advantage to using $\dot{Q}$ to estimate $\dot{V}O_2$ over f_H is that $\dot{Q}$ enables both f_H and Vs to be estimated. Assumptions that still must be taken into account when using $\dot{Q}$ to estimate metabolism include: EO_2 remains stable or varies systematically as $\dot{Q}$ changes (Webber et al. 1998), cutaneous oxygen absorption is insignificant, all metabolic events are aerobically fuelled and hematocrit and hemoglobin levels remain constant (Gallaugher and Farrell 1998).

A physiological variable that is a reliable predictor of $\dot{V}O_2$ yields a calibration curve that describes its relationship with $\dot{V}O_2$ and the fish's scope for activity in a consistent manner. Hence, the relationship between $\dot{Q}$ and $\dot{V}O_2$ must be linear or log linear, contain limited variability and be stable under environmentally and physiologically relevant conditions (Thorarensen et al. 1996b). Upon the examination of the relationship between $\dot{V}O_2$, $\dot{Q}$ and EO_2 in rainbow trout, Brodeur et al. (2001a) concluded that $\dot{Q}$ offered few advantages over f_H as a predictor of metabolic rate. The contributions of EO_2 and $\dot{Q}$ to $\dot{V}O_2$ varied with temperature and swimming activity, hence more than one regression line was needed to explain their interrelationship. Additionally, the correlation between $\dot{Q}$ and $\dot{V}O_2$ was often variable and weak. Nonetheless, $\dot{Q}$ is a close correlate of metabolic rate in Atlantic cod (Webber et al. 1998). Low-frequency metabolic events such as spontaneous pectoral and caudal fin beats are even detected, allowing researchers to accurately determine overall metabolic costs. The findings of Webber et al. (1998) have been given more weight than the concerns raised by Brodeur et al. (2001a), as various researchers have used $\dot{Q}$ to infer changes in metabolism in fish species exposed to a wide range of environmental conditions.

Toxicant Exposure

Estimating the metabolic costs of toxicant exposure is an emerging application of $\dot{Q}$ technology given the pollution stress that many fish species are exposed to in the wild. Toxicants can act to load (increase) or limit (decrease) metabolic rate (Fry 1971). During resistance to stress (Selye 1973), the energetic requirements of initiating and maintaining defence mechanisms, managing internalized toxicants (ie: catabolism, neutralization, transportation and excretion) and repairing toxicant-induced tissue damages are generally predicted to cause metabolic loading (Calow 1991). An elevation in metabolic rate is thus expected with increasing magnitude or duration of toxicant exposure until the onset of irreversible pathological effects that impair metabolism itself. However, metabolic

responses to toxicants are not always this straightforward. Metabolic rate can also decrease as a result of exposure to varying levels of toxicant(s) (Calow 1991; Brodeur et al. 2001b). Alterations in metabolic rate caused by the presence of pollutants can impact fish energetics so that the previously discussed bioenergetics equation could change to;

$$C = [M \pm \boldsymbol{T}] + G + W,$$

where $\boldsymbol{T}$ represents toxicant-imposed metabolic loading or limiting.

A small body of current literature has investigated the utility of $\dot{Q}$ as an indicator of metabolic stress resulting from pollutant exposure. Only a few toxicants, toxicant concentrations and species have been studied. Furthermore, the majority of studies have been conducted by the same researcher (Brodeur et al. 1999, 2001b, c), and this group has only employed Doppler flowprobes. Brodeur et al. (2001b) reported that exposure to pentachlorophenol (PCP) and tetrachloroguaiacol (TCG) did not alter $\dot{Q}$ in rainbow trout, although $\dot{V}O_2$ changed significantly following 24-hour exposure. In another experiment, Brodeur et al. (2001c) discovered that Atlantic salmon locomotive behaviour and blood glucose levels were more sensitive endpoints of exposure to acidic water and aluminum than $\dot{Q}$. Similarly, Dussalt et al. (2001) reported that $\dot{Q}$ in rainbow trout exposed to acidic water and aluminum did not consistently respond to toxicant exposure, whereas blood ions, metabolites and other blood parameters had well-defined responses to the treatment. Brodeur et al. (1999) found that $\dot{Q}$ was suitable for detecting lethal levels of aluminium and acidic water; adult Atlantic salmon suffered from a depression in $\dot{Q}$ accompanied by concomitant elevations in f_H and a lower Vs approximately 4 hours prior to mortality. In contrast to the above, Linton (2003) reported that routine $\dot{Q}$ consistently increased by 7 to 10% in adult Atlantic salmon exposed to thermomechanical pulp and paper mill effluent at concentrations of 12 and 25%. Moreover, $\dot{Q}$ responded reasonably quickly to the effluent, as significant increases in $\dot{Q}$ occurred within a 6-hour exposure period.

Aquaculture

Cardiac output has been used to determine the impact of digenean flukes, *Apatemon gracilis*, that are encysted in the pericardial cavity of farmed rainbow trout (Tort et al. 1987). The fluke did not cause direct mortality, but there were concerns that it was affecting cardiac performance. Using perfused heart preparations, Tort et al. (1987) discovered that $\dot{Q}$ in fish with infected hearts was only 20-40% of the $\dot{Q}$ in uninfected fish, which was almost entirely attributed to a decrease in Vs. This drastic reduction in $\dot{Q}$ could have serious repercussions to the aquaculture industry, as reduced oxygen transport to the tissues and a depressed metabolic scope could cause lower food intake, growth rates and activity (Tort et al. 1987).

Sea lice infections are also a concern in aquacultured as well as wild fish species such as Atlantic salmon, whose migratory routes span areas where intensive aquaculture is practiced. Wagner et al. (2003) studied the effects two different levels of sea lice infection on $\dot{Q}$, swimming performance and blood osmolality in Atlantic salmon. When swimming at their critical swimming speed, $\dot{Q}$ increased significantly in salmon infected with no lice and the lowest lice treatment while $\dot{Q}$ in salmon infected with the highest density of sea lice did not increase significantly and U_{crit} was 19% lower than in control fish. The bioenergetics budget of fish is again implicated in the observed response, as it is possible that fish with a higher sea lice density lost energy due to the increasingly compromised osmoregulation (Wagner et al. 2003).

Capture and Handling

Electrofishing: Cardiac output technology has been used to monitor the cardiovascular effects of electrofishing on rainbow trout (Schreer *et al.* 2004). During electroshock cardiac arrest prevailed whereas post-electroshock $\dot{Q}$ increased almost 2-fold and did not return to resting levels for 2 to 3 hours. Longer shock duration's caused the length of cardiac arrest as well as the $\dot{Q}$ recovery time to increase. Cardiac arrest causes a depression in aerobic metabolism and an oxygen debt, while the elevated $\dot{Q}$ observed post-electroshock could also cause a reduction in metabolic scope (Schreer et al. 2004).

Catch-and-Release Angling: The metabolic effects of various activities associated with catch-and-release angling have been monitored in fish through the measurement of $\dot{Q}$. Schreer et al. (2001) subjected smallmouth bass (*Micropterus dolomieu*) to short (20 s) or long (120-180 s) burst swimming events at temperatures of 12, 16 and 20°C; this protocol was designed to mimic the forced swimming that fish experience during angling. Cardiac output increased two-fold following forced swimming and did not return to resting levels for approximately 1 hour in fish that were briefly 'angled' and 2 hours in those exposed to the lengthier angling simulation. The magnitude of the cardiac response did not increase with angling duration, but cardiac recovery times did. Another interesting finding was that cardiac recovery times were not influenced significantly by water temperature (Schreer et al. 2001).

Fish with different levels of winter activity were similarly subject to an exhaustive swimming protocol at water temperatures typical of North American winters (3°C). The anaerobic exercise was intended to simulate a recreational catch-and-release angling event or other conditions that fish may experience in the winter that necessitate aerobic swimming, such as predator avoidance, food acquisition and adjustment to ice dynamics or variable water flows (Cooke et al. 2003a). Examination of basal $\dot{Q}$, $\dot{Q}$ recovery time and the magnitude of change in $\dot{Q}$ between the three fish species illustrated that cardiovascular differences existed between fish with different levels of winter activity

and highlighted the need for management decisions to recognise the unique low-temperature exercise physiology of fish (Cooke et al. 2003a).

Angling and unhooking have also been examined for their cumulative impacts on the magnitude of change in $\dot{Q}$ and the cardiac recovery time. Cooke et al. (2001) simulated exhaustive angling by subjecting rock bass (*Ambloplites rupestris*) to 30 seconds of exhaustive exercise then to either 30 or 180 seconds of air exposure. Cardiac output increased roughly 2-fold during simulated angling and took approximately twice as long (2 versus 4 hours) to recover to resting levels in fish exposed to air for a longer duration.

Additionally, $\dot{Q}$ has been applied in the examination of the metabolic effects of numerous other aspects of catch-and-release angling. For example, when the number of smallmouth bass held in a live-well increases beyond one individual, $\dot{Q}$ fails to fall to pre-angling levels within a 6-hour monitoring period, compared to 1 hour for a single bass (Cooke et al. 2002c). The use of salt or a commercial water conditioner more than doubles the length of time required for $\dot{Q}$ and its associated parameters to normalise post-angling in smallmouth bass relative to bass held in flow-through conditions of fresh lake water (Cooke et al. 2002c). In another experiment by Cooke et al. (2002c), a sequence of events similar to that which a bass may experience during a tournament were simulated. The sequence included angling, air exposure, live-well detainment with up to 4 fish, culling of the other fish from the live-well, removal from the live-well and finally, weigh-in. The initial angling and air exposure induced the most extreme cardiac disturbance (75% increase in $\dot{Q}$) and the final air exposures also contributed to an increase in $\dot{Q}$. Following the entire regime, $\dot{Q}$ took 3 hours to recover to resting levels, which was 3-times longer than it took in fish that were only angled and held in a live-well (Cooke et al. 2002c).

Furimsky et al. (2003) researched the effects of hypoxia on $\dot{Q}$ and other respiratory and cardiovascular variables to test the hypothesis that smallmouth bass were less tolerant of live-release angling events than largemouth bass. Bass are potentially subject to hypoxic water at various stages throughout a fishing tournament. Indeed, the decrease in $\dot{Q}$ during hypoxia was more pronounced in small versus largemouth bass, illustrating (along with the other results) that smallmouth bass were more sensitive to hypoxia than largemouth bass (Schreer et al. 2001).

The results of the above studies illustrate that fish subject to catch-and-release angling likely experience a period of altered and most commonly reduced metabolic scope that may contribute to increased mortality, nest abandonment, nest or self-predation and decreased parental care (Schreer et al. 2001). Catch-and-release angling is a fisheries management issue that has benefited from studies of $\dot{Q}$ recovery times. Management recommendations derived from these studies, which focus on enhanced

survival and reduced sublethal disturbances, are disseminated to fishers by conservation organisations and the government (Cooke et al. 2002a).

Predation

Cardiac output technology has enabled the energetic expenditure associated with predation attacks to be explored. Cardiac output in largemouth bass (*Micropterus salmoides*) increases by between 15-45% following simulated avian predation attempts, with smaller fish generally experiencing greater $\dot{Q}$ increases. It takes between 20 and 40 minutes for $\dot{Q}$ to recover to resting levels after a predation scare. The increase in $\dot{Q}$ suggests that predation attempts cause an increase in metabolism, which contributes to decreases in energy allocation to other components of the bioenergetics budget equation such as growth (Cooke et al. 2003b). Furthermore, it is probable that fish have less metabolic scope to escape from predators or deal with other stressors following predation attempts (Cooke et al. 2003b).

AUTOMATED BIOLOGICAL MONITORING

Automated biological monitoring (biomonitoring) is defined as a self-acting or self-regulating means of assessing water quality or chemical toxicity using living organisms (Gruber 1988). It offers many advantages over more traditional methods of assessing toxicity, such as laboratory-based bioassays, which are not amenable to real-time water quality monitoring or pollution prevention. Biomonitoring offers a quick, reliable, continuous and automated method of evaluating changes in the quality of drinking water, industrial discharges and wastewater streams (Diamond et al. 1988; Gruber 1988). Biomonitoring systems using fish currently assess changes in locomotor activity and ventilatory behaviour in automated, self-regulating, land-based chambers that are continually supplied with water from the source under assessment. The biological response variable(s) of interest is detected as or converted to an electrical signal that is transmitted to a computer. Using appropriate software, the computer recognises signals representative of 'normal' and 'abnormal' conditions. If 'abnormal' conditions are detected, it is assumed that the fish are being exposed to and affected by polluted water; an alarm is immediately activated or the appropriate corrective action is taken (Diamond et al. 1988; Gruber 1988; Biological Monitoring Inc. 2003). Biomonitoring has primarily focused on assessing changes in water quality and has been less concerned with the direct effects of xenobiotics on aquatic fauna. Changes in locomotion and ventilatory frequency can be extrapolated to predict ecological effects, however the fish species used for biomonitoring are often not native to the threatened watercourse. In addition, alterations in locomotor behaviour and ventilatory frequency are not superior predictors of fish health. Using a response variable such as $\dot{Q}$ in biomonitoring applications would allow for fluctuations in water quality as well as cardiovascular physiology and metabolic rate to be concurrently monitored. Cairns and van der Schalie (1980) proposed a list of criteria that automated biological monitoring systems should possess. These criteria

should be considered in the development of $\dot{Q}$ as a biomonitoring tool, but are beyond the scope of the current discussion (refer to Linton, 2003 for review).

CHEMICAL UPTAKE AT THE GILLS

Understanding the uptake and accumulation of waterborne chemicals and antibiotics in fish is a key area of concern to regulatory bodies, the aquaculture industry and frequent fish consumers. Both chemical and biological factors dictate the rate of chemical uptake at the gills. The flow-limited model of gill uptake, however, is solely based upon the dominant biological factors influencing chemical uptake – flow limitations on either the blood or water side of the gills (Erickson and McKim 1990). Cardiac output technology has enabled researchers to study blood flow to the gills and consequently validate this flow-limited model. Through the use of $\dot{Q}$ technology, researchers have gained a deeper understanding of the uptake of various organic chemicals at the gills of fish (Schmieder and Weber 1992; McKim et al. 1994).

CONCLUSION

Cardiac output in fish can be measured both directly and indirectly. Flowprobes that measure $\dot{Q}$ directly have largely replaced outdated techniques such as the indicator dilution method and the Fick principle, however the research objectives, fish species under examination, as well as equipment cost and availability dictate which $\dot{Q}$ measurement technique is employed. Employing $\dot{Q}$ technology in disciplines including bionergetics, toxicology, aquaculture, catch-and-release angling, biomonitoring and chemical uptake at the gills demonstrate the utility of this tool for use in various unique applications. The development of a telemetry tag that measures $\dot{Q}$ will be a wide-sweeping and significant contribution to the study of fish. This paper has provided an overview of $\dot{Q}$ measurement, the benefits and challenges associated with each measurement technique and the present-day applications of $\dot{Q}$ technology.

REFERENCES

Adams, S.M. and Breck, J.E. 1990. Bioenegetics. *In* Methods for Fish Biology. Edited by C.B. Shreck and P.B. Moyle. Am. Fish. Soc. Maryland. pp. 389-415.

Axelsson, M. 2004. Michael Axelsson - Personal Home Page. http://vivaldi.zool.gu.se/PersonalPages/MichaelA/MichaelAxelsson.htm (accessed April 13, 2004).

Axelsson, M. and Farrell, A.P. 1993. Coronary blood flow *in vivo* in the coho salmon (*Oncorhynchus kitsutch*). Am. Physiol. Soc. 44: 963-971.

Axelsson, M., Davison, W., Forster, M.E. and Farrell, A.P. 1992. Cardiovascular responses of the red-blooded Antarctic fishes, *Pagothenia bernacchii* and *P. borchgrevinki*. J. Exp. Biol. 167: 179-201.

Axelsson, M., Thorarensen, H., Nilsson, S. and Farrell, A.P. 2000. Gastrointestinal blood flow in the red Irish lord, *Hemilepidotus hemilepidotus*: long-term effects of feeding and adrenergic control. J. Comp. Physiol. B: 170: 145-152.

Barron, M.G., Tarr, B.D. and Hayton, W.L. 1987. Temperature-dependence of cardiac output and regional blood flow in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 31: 735-744.

Biological Monitoring Inc. 2003. BioSensor® : automated and continuous water quality monitoring. http://www.biomon.com/biosenso.html (accessed 14 April, 2003).

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21: 1183-1226.

Brodeur, J.C., Ytestøyl, T., Finstad, B. and McKinley, R.S. 1999. Increase of heart rate without elevation of cardiac output in Atlantic salmon (*Salmo salar*) exposed to acidic water and aluminum. Can. J. Fish. Aquat. Sci. 56: 184-190.

Brodeur, J.C., Dixon, D.G. and McKinley, R.S. 2001a. Assessment of cardiac output as a predictor of metabolic rate in rainbow trout. J. Fish Biol. 58: 439-452.

2001b. Inhibition of oxygen consumption by pentachlorophenol and tetrachloroguaiacol in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 54: 143-148.

Brodeur, J.C., Økland, F., Finstad, B., Dixon, D.G. and McKinley, R.S. 2001c. Effects of subchronic exposure to aluminium in acidic water on bioenergetics of Atlantic salmon (*Salmo salar*). Ecotoxicol. Env. Safety 49: 226-234.

Bunt, C.M., Cooke, S.J., Schreer, J.F. and Philipp, D. 2004. Effects of incremental increases in silt load on the cardiovascular performance of riverine and lacustrine rock bass, *Ambloplites rupestris*. Environ. Poll. 128: 437-444.

Bushnell, P.G., Brill, R.W. and Bourke, R.E. 1990. Cardiorespiratory responses of skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*Thunnus obesus*) to acute reductions of ambient oxygen. Can. J. Zool. 68: 1857-1865.

Cairns, J. and van der Schalie, W.H. 1980. Biological monitoring Part I: early warning systems. Water Res. 14: 1179-1196.

Calow, P. 1991. Physiological costs of combating chemical toxicants: ecological implications. Comp. Biochem. Physiol. C 100: 3-6.

Cech, J.J. 1990. Respirometry. *In* Methods for Fish Biology. Edited by C.B. Shreck and P.B. Moyle. American Fisheries Society, Maryland. pp. 335-362.

Cooke, I.R. 1980. Functional aspects of the morphology and vascular anatomy of the gills of the endeavour dogfish, *Centrophorus scalpratus* (McCullock) (Elasmobranchii: Squalidae). Zoomorphologie 94: 167-183.

Cooke, I.R. and Campbell, G. 1980. The vascular anatomy of the gills of the smooth toadfish, *Torquiginer glaber* (Teleostii: Tetraododontidae). Zoomorphologie 94: 151-166.

Cooke, S.J., Philipp, D.P., Dunmall, K.M. and Schreer, J.F. 2001. The influence of terminal tackle on injury, handling time and cardiac disturbance of rock bass. North Am. J. Fish. Manage. 21: 333-342.

Cooke, S.J. Schreer, J.F., Wahl, D.H., Philipp, D.P., Suski, C.D. and Tufts, B.L. 2002a. Interfacing cardiac physiology with fisheries management through studies of catch-an-release angling effects on freshwater recreational fishes. *In* Cardiovascular Physiology of Fish, Proceedings of the International Congress on the Biology of Fish. Edited by K. Gamperl, T. Farrell and D. MacKinlay. Vancouver, British Columbia. 122 p. http://www-heb.pac.dfo-mpo.gc.ca/congress/2002/Cardiovasc/Cooke.pdf (accessed 30 March, 2004).

Cooke, S.J., Schreer, J.F., Dunmall, K.M. and Philipp, D.P. 2002b. Strategies for Quantifying Sublethal Effects of Marine Catch-and-Release Angling: insights from Novel Freshwater Applications. Am. Fish. Soc. Symp. 30: 121-134.

Cooke, S.J., Schreer, J.F., Wahl, D.H. and Philipp, D.P. 2002c. Physiological impacts of catch-and-release angling practices on largemouth bass and smallmouth bass. Am. Fish. Soc. Symp. 31: 489-512.

Cooke, S.J., Grant, E.C., Schreer, J.F., Philipp, D.P. and Devries, A.L. 2003a. Low temperature cardiac response to exhaustive exercise in fish with different levels of winter quiescence. Comp. Biochem. Physiol. A 134: 157-165.

Cooke, S.J., Steinmetz, J., Degner, J.F., Grant, E.C. and Philipp, [illegible] 2003b. Metabolicfright responses of different-sized largemouth [illegible] (*Micropterus salmoides*) to two avian predators show variations in nonlethal energetic costs. Can. J. Zool. 81: F699-709.

Cousins, K.L. and Farrell, A.P. 1996. Stretch-induced release of atrial natriuretic factor from the heart of rainbow trout (*Oncorhynchus mykiss*). Can. J. Zool. 74: 380-387.

Crocker, C.E., Farrell, A.P., Gamperl, A.K. and Cech, J.J. 2000. Cardiorespiratory responses of white sturgeon to environmental hypercapnia. Am. J. Physiol. 279: R617-R628.

Davie, P.S. and Farrell, A.P. 1991. Cardiac performance of the isolated heart preparation from the dogfish (*Squalus acanthias*): the effects of hypoxia and coronary artery perfusion. Can. J. Zool. 69: 1822-1828.

Davie, P.S. and Forster, M.E. 1980. Cardiovascular responses to swimming in eels. Comp. Biochem. Physiol. A 67: 367-373.

Davie, P.S. and Franklin, C.E. 1992. Myocardial oxygen consumption and mechanical efficiency of a perfused dogfish heart preparation. J. Comp. Physiol. B 162: 256-262.

Davie, P.S., Farrell, A.P. and Franklin, C.E. 1992. Cardiac performance of an isolated eel heart: effects of hypoxia and coronary artery perfusion. J. Exp. Zool. 262: 113-121.

Daxboeck, C., Davie, P.S., Perry, S.F. and Randall, D. 1982. Oxygen uptake in a spontaneously ventilating, blood-perfused trout preparation. J. Exp. Biol. 101: 35-45.

Diamond, J., Collins, M. and Gruber, D. 1988. An overview of automated biomonitoring–past developments and future needs. *In* Automated biomonitoring: living sensors as environmental monitors. Edited by D.S. Gruber and J.M. Diamond. Ellis Horwood, New York. pp. 23-39.

Drost, C.J. 1978. Vessel diameter-independent volume flow measurements using ultrasound. *In* Proceedings of the San Diego Biomedical Symposium, San Diego, California. San Diego Biomedical Symposium, Vol. 17. pp. 299-302.

Dunel, S. and Laurent, P. 1980. Functional organisation of the gill vasculature in different classes of fish. *In* Epithelial Transport in the Lower Vertebrates. Edited by B. Lahlou. Cambridge University Press, United Kingdom. pp. 37-58.

Dussault, E.B., Playle, R.C., Dixon, D.G. and McKinley, R.S. 2001. Effects of sublethal, acidic aluminum exposure on blood ions and metabolites, cardiac output, heart rate, and stroke volume of rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. 25: 347-357.

Erickson, R.J. and McKim, J.M. 1990. A simple flow-limited model for exchange of organic chemicals at fish gills. Environ. Toxicol. Chem. 9: 159-165.

Farrell, A.P. 1984. A review of cardiac performance in the teleost heart: Intrinsic and humoral regulation. Can. J. Zool. 62: 523-536.

1991. From hagfish to tuna: a perspective on cardiac function in fish. Physiol. Zool. 64: 1137-1164.

Farrell, A.P. and Jones, D.R. 1992. The Heart. *In* Fish Physiology, Volume XIIA. Edited by W.S. Hoar, D.J. Randall and A.P. Farrell. Academic Press Inc., San Diego. pp. 1-88.

Farrell, A.P., MacLeod, K.R. and Driedzic, W.R. 1982. The effects of preload, after load and epinephrine on cardiac performance in the sea raven, *Hemitripterus americanus*. Can. J. Zool. 60: 3165-3171.

Farrell, A.P., MacLeod, K.R., Driedzic, W.R. and Wood, S. 1983. Cardiac performance in the *in situ* perfused fish heart during extracellular acidosis: interactive effects of adrenaline. J. Exp. Biol. 107: 415-429.

Farrell, A.P., MacLeod, K.R. and Chancey, B. 1986. Intrinsic mechanical properties of the perfused rainbow trout heart and the effects of catecholamines and extracellular calcium under control and acidotic conditions. J. Exp. Biol. 125: 319-345.

Farrell, A.P., Johansen, J.A. and Graham, M.S. 1988. The role of the pericardium in cardiac performance of trout (*Salmo gairdneri*). Physiol. Zool. 61: 213-221.

Farrell, A.P., Johnasen, J.A. and Suarez, R.K. 1991. Effects of exercise-training on cardiac performance and muscle enzymes in rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. 9: 303-312.

Farrell, A.P., Small, S. and Graham, M.S. 1989. Effect of heart rate and hypoxia on the performance of a perfused trout heart. Can. J. Zool. 67: 274-280.

Farrell, A.P., Davie, P.S., Franklin, C.E., Johansen, J.A. and Brill, R.W. 1992. Cardiac physiology in tunas. 1. *In vitro* perfused heart preparations from yellowfin and skipjack tunas. Can. J. Zool. 70: 1200-1210.

Farrell, A.P., Lee, C.G., Tierney, K., Hodaly, A., Clutterham, S., Healey, M., Hinch, S. and Lotto, A. 2003. Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. J. Fish. Biol. 62: 64-84.

Franklin, C.E. and Davie, P.S. 1993. The role of the pericardium in cardiac function in the dogfish, *Squalus acanthias*. J. Fish. Biol. 43: 213-219.

Fry, F.E. 1971. The Effect of Environmental Factors on the Physiology of Fish. *In* Fish physiology, Vol. VI: environmental relations and behaviour. Edited by W.S. Hoar and D.J. Randall. Academic Press, United Kingdom. pp. 1-98.

Furimsky, M., Cooke, S.J., Suski, C.D., Wang, Y and Tufts, B.L. 2003. Respiratory and circulatory responses to hypoxia in largemouth bass and smallmouth bass: implications for "live-release" angling tournaments. Trans. Am. Fish. Soc. 132: 1065-1075.

Gallaugher, P. and Farrell, A.P. 1998. Hematocrit and blood oxygen-capacity. *In* Fish respiration, fish physiology series VXVII. Edited by S.F. Perry and B. Tufts. Academic Press, San Diego. pp. 185-219.

Gamperl, A.K., Pinder, A.W. and Boutilier, R.G. 1994. Effect of coronary ablation and adrenergic stimulation on *in vivo* cardiac performance in trout (*Oncorhynchus mykiss*). J. Exp. Biol. 186: 127-143.

Graham, M.S. and Farrell, A.P. 1989. The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. Physiol. Zool. 62: 38-61.

Gruber, D. 1988. A historical perspective. *In* Automated biomonitoring: living sensors as environmental monitors. Edited by D.S. Gruber and J.M. Diamond. Ellis Horwood, New York. pp. 15-22

Guyton, A.C., Jones, C.E. and Coleman, T.G. 1973. Circulatory physiology: cardiac output and its regulation, 2nd edition. W.B. Saunders, Philadelphia. 468 p.

Hemmingsen, E.A. and Douglas, E.L. 1970. Respiratory characteristics of the haemoglobin-free fish *Chaenocephalus aceratus*. Comp. Biochem. Physiol. 32: 733-744.

Hinch, S.G., Diewert, R.E., Lissimore, T.J., Prince, A.M., Healy, M.C. and Henderson, M.A. 1996. Use of electromyogram telemetry to assess difficult passage areas for river-migrating adult sockeye salmon. Trans. Am. Fish. Soc. 125: 253-260.

Holeton, G.F. and Randall, D.J. 1967. The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J. Exp. Biol. 46:317-327.

Hughes, G.M., Peyraud, C., Peyraud-Waitzenegger, M. and Soulier, P. 1982. Proportion of cardiac output concerned with gas exchange in the gills of the eel (*Anguilla anguilla*). J. Physiol. 310: 61-62.

Iowa Doppler Products. 2003. Iowa Doppler Products. http://soli.inav.net/~idp/. (accessed 23 October, 2003).

Johansen, K. and Peterson, K. 1981. Gill O_2 consumption in a teleost fish, *Gadus morhua*. Resp. Physiol. 44: 277-284.

Johnstone, A.D., Lucas, M.C., Boylan, P. and Carter, T. 1992. Telemetry of tail beat frequency in Atlantic salmon, *Salmo salar* L., during spawning . *In* Wildlife telemetry: remote monitoring and tracking of animals. Edited by I.M. Priede and S.M. Swift. Ellis Horwood, New York. pp.456-465.

Keen, J.E. and Farrell, A.P. 1994. Maximum prolonged swimming speed and maximum cardiac performance of rainbow trout, *Oncorhynchus mykiss*, acclimated to two different water temperatures. Comp. Biochem. Physiol. 108A: 287-295.

Kicenuik, J.W. and Jones, D.R. 1977. The oxygen transport system in trout *(Salmo gairdneri)* during sustained exercise. J. Exp. Biol. 69: 247-260.

Kirsch, R. and Nonnotte, G. 1977. Cutaneous respiration in three fresh water teleosts. Resp. Physiol. 29: 339-354.

Kolok, A.S., Spooner, R.M. and Farrell, A.P. 1993. The effect of exercise on the cardiac output and blood flow distribution of the largescale sucker *Catostomus macrocheilus*. J. Exp. Biol. 183: 301-321.

Korsmeyer, K.E., Lai, N.C., Shadwick, R.E. and Graham, J.B. 1997. Heart rate and stroke volume contributions to cardiac output in swimming yellowfin tuna: response to exercise and temperature. J. Exp. Biol. 200: 1975-1986.

Linton, E.D. 2003. Physiological effects of thermomechanical newsprint mill effluent on adult Atlantic salmon from the Exploits River, Newfoundland, Canada. Thesis (MSc.) University of British Columbia, Vancouver, BC. 143 p.

Lucas, M.C. 1994. Heart rate as an indicator of metabolic rate and activity in adult Atlantic slamon, *Salmo salar*. J. Fish Biol. 44: 889-903.

Metcalfe, J.D. and Butler, P.J. 1982. Difference between directly measured and calculated values for cardiac output in the dogfish: a criticism of the Fick method. J. Exp. Biol. 99: 255-268.

McKim, J.M., Nicholas, J.W., Lien, G.J. and Bertelsen, S.L. 1994. Respiratory-cardiovascular physiology and chlorethane gill flux in the channel catfish, *Ictalurus puntatus*. J. Fish Biol. 44: 527-547.

Neumann, P. Holeton, G.F. and Heisler, N. 1983. Cardiac output and regional blood flow in gills and muscles after exhaustive exercise in rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 105: 1-14.

Nichols, W.W. and O'Rourke, M.F. 1990. McDonald's blood flow in arteries: theoretic, experimental and clinical principles, 3RD Edition. Lea and Febiger, Philadelphia, USA. 456 p.

Nonnotte, G. and Kirsch, R. 1978. Cutaneous respiration in seven sea-water teleosts. Resp. Physiol. 35: 111-118.

Olson, K.R. and Kent, B. 1980. The microvasculature of the elasmobranch gill. Cell Tissue 209: 49-63.

Perry, S.F. and Farrell, A.P. 1989. Perfused preparations in comparative respiratory physiology. *In* Techniques in comparative respiratory physiology: an experimental approach. Edited by C.R. Bridges and P.J. Butler. Cambridge University Press, London, England. pp. 223-257.

Randall, D. 1985. Shunts in fish gills. *In* Cardiovascular shunts – phylogenetic, ontogenetic and clinical aspects. Edited by K. Johansen and W. Burggren. Presented at the Alfred Benson Symposium. Munksgaard, Copenhagen, Denmark. pp. 71-87.

Reneman, R.S. and Hoeks, A. 1977. Continuous wave and pulsed Doppler flowmeters – a general introduction. *In* Echocardiology. Edited by. N. Born. Marinu Nijhoff, the Hague, The Netherlands. pp. 189-203.

Richards, B.D. and Fromm, P.O. 1969. Patterns of blood flow through filaments and lamellae of isolated-perfused rainbow trout gills. Comp. Biochem. Physiol. 29: 1063-1070.

Rogers, S.C. and Weatherly, A.H. 1983. The use of opercular muscle electromyograms as an indicator of the metabolic costs of fish activity in rainbow trout, *Salmo gairdneri* Richardson, as determined by radiotelemetry. J. Fish Biol. 23: 535-547.

Santer, R.M. 1985. Morphology and innervation of the fish heart. Adv. Anat. Embryol. Cell Biol. 89: 1-97.

Selye, H. 1973. The evolution of the stress concept. Am. Sci. 61: 692-699.

Schmieder, P.K. and Weber, L.J. 1992. Blood and water flow limitation on gill uptake of organic chemicals in the rainbow trout. Aquat. Toxicol. 24: 103-122.

Schreer, J.F. and Cooke, S.J. 2002. Behavioral and physiological responses of smallmouth bass to a dynamic thermal environment. Am. Fish. Soc. Symp. 31: 191-203.

Schreer, J.F., Cooke, S.J. and McKinley, R.S. 2001. Cardiac response to variable forced exercise at different temperatures: an angling simulation for smallmouth bass. Trans. Am. Fish. Soc. 130: 783-795.

Schreer, J.F., Cooke, S.J. and Connors, K.B. 2004. Electrofishing-induced cardiac disturbance and injury in rainbow trout. J. Fish Biol. 64:1-19.

Shima, T. and Namba, K. 1996. Roles of the pectoral musculoskeletal framework and the pericardium in the cardiac function of carp. Fish. Sci. 62: 715-718.

Stecyk, J.A. and Farrell, A.P. 2002. Cardiorespiratory responses of the common carp (*Cyprinus carpio*) to severe hypoxia at three acclimation temperatures. J. Exp. Biol. 205: 759-768.

Thorarensen, H., Gallaugher, P.E., Kiessling, A.K. and Farrell, A.P. 1993. Intestinal blood flow in swimming chinook salmon *Oncorhynchus tshawytscha* and the effects of heamatocrit on blood flow distribution. J. Exp. Biol. 179: 115-129.

Thorarensen, H., Gallaugher, P. and Farrell, A.P. 1996a. Cardiac output in swimming rainbow trout, *Oncorhynchus mykiss*, acclimated to seawater. Physiol. Zool. 69: 139-153.

Thorarensen, H., Gallaugher, P.E. and Farrell, A.P. 1996b. The limitations of heart rate as a predictor of metabolic rate in fish. J. Fish Biol. 49: 226-236.

Tort, L., Watson, J.J. and Priede, I.G. 1987. Changes in *in vitro* heart performance in rainbow trout, *Salmo gairdneri* Richardson, infected with *Apatemon gracilis* (Digenea). J. Fish Biol. 30: 341-347.

Transonic Systems Inc. 1999. Theory of operation: transit time ultrasound technology. Copyright Transonic Systems Inc., USA. TN30-1 Rev. 11/99.

Transonic Systems Inc. 2003. Blood flow measurements in cardiac surgery. www.transonic.com/Surgical_Clinical Home/surgical_clinical_home.html (accessed 23 October, 2003).

Vogel, W., Vogel, V. and Pfautsch, M. 1976. Arterio-venous anatomises in rainbow trout gill filaments. Cell Tissue Res. 167: 373-385.

Wagner, G.N., McKinley, R.S., Bjørn, P.A. and Finstad, B. 2003. Physiological impact of sea lice on swimming performance of Atlantic salmon. J. Fish Biol. 62: 1000-1009.

Webber, D.M., Boutilier, R.G. and Kerry, S.R. 1998. Cardiac output as a predictor of metabolic rate in cod, *Gadus morhua*. J. Exp. Biol. 201: 2779-2789.

Widdows, J. and Donkin, P. 1991. Role of physiological energetics in ecotoxicology. Comp. Biochem. Physiol. C 100: 69-75.

Young, A.H., Tytler, P., Holliday, F.G. and MacFarlane, A. 1972. A small sonic tag for measurement of locomotor activity in fish. J. Fish Biol. 4: 57-65.